

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Michael Knoblauch, et al.  
Serial No: 10/605,104  
Filing Date: 9/9/2003  
Title: Forisomes, Method for Their Isolation, and Their Use as a  
Molecular Working Machine  
Examiner: Marsha M. Tsay  
Art Unit: 1656

**Commissioner for Patents**  
**Alexandria, VA 22313-1450**

**DECLARATION PURSUANT TO 37 CFR 1.132**

I, Prof. Dr. Dirk Prüfer, Machabaerstr. 57, Cologne, Germany, am one of the co-inventors of the instant application. I received my PhD degree ("Dr.") in 1992 at the University of Cologne and was Head of the Department "Functional and Applied Genomics" and Head of the Section "Molecular Biology" at the assignee, Fraunhofer-Gesellschaft, at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Aachen and Schmollenberg, Germany, between 1999 and 2004. As from December 2004, I am Full Professor for Molecular Biotechnology of Plants at the Institute for Biochemistry and Biotechnology of Plants at Münster University, as well as Head of Department "Functional and Applied Genomics" at the above Fraunhofer Institute.

The main field of my scientific research is directed to forisomes since 2000.

The amino acid sequence SEQ ID NO:2 as described in our above mentioned application is a sequence which occurs highly conserved in all P1 proteins. These proteins are responsible for the mechanical behavior ("stopcock") of the forisomes in fabaceae. In order to demonstrate that SEQ ID NO:2 occurs in and therefore is an inherent feature of all P1 proteins, i.e. that it can be assigned to the P1 protein only, the amino acid sequence of five P1 proteins, derived from four different fabaceae plants, is listed on the attached sheet. As examples, EGFDIAFK = Glu-Gly-Phe-Asp-Ile-Ala-Phe-Lys is found in line 11 of each of the sequences; these five sequences are publicly accessible via the US NCBI gene database:

MtP1.1 = 1. Variant of Medicago trunculata = MtSEO-F1

<http://www.ncbi.nlm.nih.gov/protein/ABV32455.1>

MtP1.2 = 2. Variant of *Medicago trunculata* = MtSEO-F4

<http://www.ncbi.nlm.nih.gov/protein/ADN32805.1>

GmP1 = *Glycine max.* = GmSEO-F4

<http://www.ncbi.nlm.nih.gov/protein/ADN32792.1>

VfP1 = *Vicia faba* = Vffor1

<http://www.ncbi.nlm.nih.gov/protein/ABV32453.1>

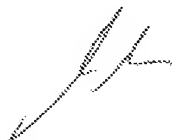
CgP1 = *Canavalia gladiata* = Cgfor1

<http://www.ncbi.nlm.nih.gov/protein/ABV32453.1>

About the forisomes of *Medicago trunculata*, I have reported with co-authors in G.A.Noll et al., *Plant Mol Biol* (2007), 65:285-294 (copy attached). The provisional name of the protein as used in our patent application ("P1") had been corrected therein to be in line with systematic designations used in this field; however, since the term "for" was already occupied, it was later amended into "SEO" ("Sieve Element Occlusion"), see the paper e.g. B. Müller et al. in *Appl. Microbiol. Biotechnol.* (2010) 88:689-698 (copy attached), where we report on the possibility to obtain recombinant artificial forisomes from expression of MtSEO1 and MtSEO4 in plants and yeast.

I herewith declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and that I am warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Signed this \_\_\_\_4\_\_\_\_ day of October 2010



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(signature)

DIRK PRÜFER  
(printed name)

Met1.1 MS-LGN-----G--KLLNFFDLDSQYLKRV  
Met1.2 MS-LSNLGS-----TATUSNLQKMTUSLQ--KA-NELPNPFLHGPQZLNKV  
Cqf1 MS-DEK-----PQCK--ALLPNPFLYLSGSELEKV  
VfP1 MS-FSNSRAARTGTLVQMGSGTMSLLIQKATSGMPPHKNMYLHGFELHDSHILETV  
GmP1 MAQLSNGT-G-----STTLYSKSTTSTPH-RA--GLPNPFDLTSDQYLDIY  
SEQ NO:2 -----

Met1.1 YLNLHDSGNOGKIVLPHLONVILKRL-----AESRAEPEPEFT  
Met1.2 YLNLHVTLSKPCNTHIYFELVSGVVLQTT-----KLSVTSFKPEFT  
Cqf1 YLHTYXDEACDRSTFLNLMSTVIMHSTRIYETFIKRPVNHGFQSGMLITSEFKPSFKK  
VfP1 YLNMPTDEEFOKATGXIPELVSTLILQNT-----QZFTGSHEDFT  
GmP1 YLNLHDSSTCDSTMLNLYNLYVLSGG-----FISAAGPYVDFLT  
SEQ NO:2 -----

Met1.1 LKLTSCQMITTEPGEKXVHQITHWILQQLKTYGNDAKALIALAAPTLEYGMLLYLTETST  
Met1.2 LKLTSCQMITTTHNDHCHQITLGLLQMLRYSNDAKALITLAAPTLEYGMLLYLTETST  
Cqf1 LKLTSCQMITTEPGLNARQITMBILQQLRPSFNGKALIALAAPTLEYGMLLYLTETST  
VfP1 LKLTSCQMITTTRGVAMCVHQTILWLQNLRYGNDAKALITLAAPTLEYGMLLYLTETST  
GmP1 LKLTSCQMITTTRGVAMCVHQTILWLQNLRYGNDAKALITLAAPTLEYGMLLYLTETST  
SEQ NO:2 -----

Met1.1 GSDQLVHSLKILNQIQIRKVTV--PSTDLVELIMDLIMINENATRSQVGYNTLEWFGLED  
Met1.2 TTTTLQNLKRLVNLQVQTRKISH--DVTLLVRYIVDMLRLNWNATWSADGVDVDFPALT  
Cqf1 PSDQLGNSLKLILNQIQIRKQIFITDYNNSVVLNMEVYVQITPNWGLWLAGYDTETYPALSD  
VfP1 ATDTEGSHLQQLGQTRKIST--GEFELVNFIVKELIMKCKNAAWSAGQDFEDVAALTE  
GmP1 TNDVLGNSLKLILNQIQIRKAS--R--VQELVMYVQVQPHINWATYAAQGYDFEDVDFLSE  
SEQ NO:2 -----

Met1.1 ALQGEIVAVYWTIASTVAATONLIGVGDYTLSDPYEKLVFVQKLAERHKLSEKQITDSVE  
Met1.2 ALQGEIVAVYWTIASTVAATONLIGVGDYTLSDPYEKLVFVQKLAERHKLSEKQITDSVE  
Cqf1 ALQGEIVAVYWTIASTVAATONLIGVGDYTLSDPYEKLVFVQKLAERHKLSEKQITDSVE  
VfP1 ALQGEIVAVYWTIASTVAATONLIGVGDYTLSDPYEKLVFVQKLAERHKLSEKQITDSVE  
GmP1 ALQGEIVAVYWTIASTVAATONLIGVGDYTLSDPYEKLVFVQKLAERHKLSEKQITDSVE  
SEQ NO:2 -----

Met1.1 EYLAKNKALSHFKXITDFKLILQ---RNG--DNLLYDSTTNETDTEVFNKDYVLLFISG  
Met1.2 EYLAKNKALSHFKXITDFKLILQ---RNGSTDYFQIYQONVQVMSGLDTFKQKHYLLFTSG  
Cqf1 EYLAKNKALSHFKXITDFKLILQ---RNGSTDYFQIYQONVQVMSGLDTFKQKHYLLFTSG  
VfP1 EYLAKNKALSHFKXITDFKLILQ---RNGSTDYFQIYQONVQVMSGLDTFKQKHYLLFTSG  
GmP1 EYLAKNKALSHFKXITDFKLILQ---RNGSTDYFQIYQONVQVMSGLDTFKQKHYLLFTSG  
SEQ NO:2 -----

Met1.1 LNFVDEELILLNBIHHLQGMFQVI--EGTKHEDFKILWIFINIV---DQKQKPEGLNKEI  
Met1.2 LNFVDEELILLNBIHHLQGMFQVI--EGTKHEDFKILWIFINIV---DQKQKPEGLNKEI  
Cqf1 LNFVDEELILLNBIHHLQGMFQVI--EGTKHEDFKILWIFINIV---DQKQKPEGLNKEI  
VfP1 LNFVDEELILLNBIHHLQGMFQVI--EGTKHEDFKILWIFINIV---DQKQKPEGLNKEI  
GmP1 LNFVDEELILLNBIHHLQGMFQVI--EGTKHEDFKILWIFINIV---DQKQKPEGLNKEI  
SEQ NO:2 -----

Met1.1 RSTAVEYFTELGLKILIR---ENLNEENKPIFVVLSEGLKGNNDGMMXIZFQKHIIDALFR  
Met1.2 RSTAVEYFTELGLKILIR---ENLNEENKPIFVVLSEGLKGNNDGMMXIZFQKHIIDALFR  
Cqf1 RSTAVEYFTELGLKILIR---ENLNEENKPIFVVLSEGLKGNNDGMMXIZFQKHIIDALFR  
VfP1 RSTAVEYFTELGLKILIR---ENLNEENKPIFVVLSEGLKGNNDGMMXIZFQKHIIDALFR  
GmP1 RSTAVEYFTELGLKILIR---ENLNEENKPIFVVLSEGLKGNNDGMMXIZFQKHIIDALFR  
SEQ NO:2 -----

Met1.1 KQDSTELTQKMKSWEDVTERVNLGIQVKGDAYIFTYGSSEKNIQDPTLELEKIKRHETI  
Met1.2 KQDSTELTQKMKSWEDVTERVNLGIQVKGDAYIFTYGSSEKNIQDPTLELEKIKRHETI  
Cqf1 KQDSTELTQKMKSWEDVTERVNLGIQVKGDAYIFTYGSSEKNIQDPTLELEKIKRHETI  
VfP1 KQDSTELTQKMKSWEDVTERVNLGIQVKGDAYIFTYGSSEKNIQDPTLELEKIKRHETI  
GmP1 KQDSTELTQKMKSWEDVTERVNLGIQVKGDAYIFTYGSSEKNIQDPTLELEKIKRHETI  
SEQ NO:2 -----

Met1.1 LRAELIIEHYHLGKDE---PPIVERFWIIESEKL--KMHQDSILKEIQHVNGLLCLNQD  
Met1.2 LRAELIIEHYHLGKDE---PPIVERFWIIESEKL--KMHQDSILKEIQHVNGLLCLNQD  
Cqf1 LRAELIIEHYHLGKDE---PPIVERFWIIESEKL--KMHQDSILKEIQHVNGLLCLNQD  
VfP1 LRAELIIEHYHLGKDE---PPIVERFWIIESEKL--KMHQDSILKEIQHVNGLLCLNQD  
GmP1 LRAELIIEHYHLGKDE---PPIVERFWIIESEKL--KMHQDSILKEIQHVNGLLCLNQD  
SEQ NO:2 -----

Met1.1 PQQWVILLKSGYHVLGKGPVYQTLADPDINKDVLMGGFELAFKYYDYTKVKDTYV-  
Met1.2 PQQWVILLKSGYHVLGKGPVYQTLADPDINKDVLMGGFELAFKYYDYTKVKDTYV-  
Cqf1 PQQWVILLKSGYHVLGKGPVYQTLADPDINKDVLMGGFELAFKYYDYTKVKDTYV-  
VfP1 PQQWVILLKSGYHVLGKGPVYQTLADPDINKDVLMGGFELAFKYYDYTKVKDTYV-  
GmP1 PQQWVILLKSGYHVLGKGPVYQTLADPDINKDVLMGGFELAFKYYDYTKVKDTYV-  
SEQ NO:2 -----

Met1.1 KQPCEITMYDNTNMGVLIATISCPNPTCGRVMEVSGVHYKCHRDAAAPQNGIT  
Met1.2 KQPCEITMYDNTNMGVLIATISCPNPTCGRVMEVSGVHYKCHRDAAAPQNGIT  
Cqf1 KQPCEITMYDNTNMGVLIATISCPNPTCGRVMEVSGVHYKCHRDAAAPQNGIT  
VfP1 KQPCEITMYDNTNMGVLIATISCPNPTCGRVMEVSGVHYKCHRDAAAPQNGIT  
GmP1 KQPCEITMYDNTNMGVLIATISCPNPTCGRVMEVSGVHYKCHRDAAAPQNGIT  
SEQ NO:2 -----